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Studies on the Mechanism of Selectivity of the Auxin Herbicide Quinmerac

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Abstract: Investigations were conducted to elucidate the mechanism of selectivity of the auxin herbicide, quinmerac, in cleavers (*Galium aparine*) and the tolerant crops sugarbeet (*Beta vulgaris*), oilseed rape (*Brassica napus*) and wheat (*Triticum aestivum*). After root treatment with the herbicide, the selectivity has been quantified as approximately 400-fold between oilseed rape and *Galium* and 1000-fold between sugarbeet or wheat and the weed species. When 1 and 10 μM [^{14}C]quinmerac were applied for 4 h, no significant differences between root absorption and translocation of ^{14}C by *Galium* and the crop species were found. After 16 h, metabolism of [^{14}C]quinmerac to the biologically inactive hydroxymethyl and dicarboxylic acid derivatives was more rapid in wheat and sugarbeet than in *Galium*. In oilseed rape, a lower rate of herbicide metabolism was observed. In *Galium*, accumulations of abscisic acid (ABA), triggered by quinmerac-stimulated ethylene biosynthesis, were found to cause the herbicidal growth inhibition which develops during 24 h of application. Within 1 h of treatment, quinmerac stimulated 1-aminocyclopropane-1-carboxylic acid (ACC) synthase activity and ACC concentration specifically in *Galium* shoot tissue. During the next 4 h, ACC synthase activity was increased up to 50-fold, relative to the control. Within 3 h of exposure to quinmerac, increased ethylene formation followed by higher ABA levels was detected. In sugarbeet, oilseed rape and wheat, quinmerac did not stimulate ACC synthase activity and ACC and ABA levels. It is suggested that (i) the selectivity of quinmerac is primarily based upon the lower sensitivity to the herbicide of the tissue/target in the crop species, (ii) the induction process of the ACC synthase activity in the shoot tissue is the primary target of herbicidal interference. In wheat and sugarbeet, tolerance to quinmerac is additionally increased by a more rapid metabolism. © 1998 SCI.

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Key words: abscisic acid (ABA); 1-aminocyclopropane-1-carboxylic acid (ACC) synthase; auxin herbicide; ethylene; quinmerac

1 INTRODUCTION

Substituted quinolinecarboxylic acids are a new class of highly selective auxin herbicides of which quinmerac (7-chloro-3-methylquinoline-8-carboxylic acid) has been introduced commercially in the past two years.[1,2] Quinmerac effectively controls important dicotyledonous weeds, such as cleavers (*Galium aparine* L.) in sugarbeet, oilseed rape and wheat.[2] In *Galium*, the compound is taken up *via* the root and leaves within a few hours of application, and is translocated systemically in the plant.[2] Quinmerac induced epinastic symptoms on stems and leaves within 5 h of root-treatment which were followed during the next 20 h by inhibition of root and particularly shoot growth, and a reduction in water consumption.[3] After two to four days, stem necrosis and chlorosis and wilting of the shoot proceeded. Based on the concentrations applied, shoot growth of *Galium* was 33-fold more sensitive to quinmerac than root growth (Grossmann, K. & Scheltrup, F. unpublished results).

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The levels of the direct biosynthetic precursor, 1-aminocyclopropane-1-carboxylic acid (ACC), and ethylene formation increased, particularly in the shoot tissue. Accumulations of abscisic acid (ABA) proceeded more slowly and reached 70 times that in untreated plants after 48 h.[3] Within 24 h of quinmerac exposure, increased ABA resulted in reductions in stomatal aperture, water consumption, photosynthetic carbon dioxide uptake and growth.[3] Thus, the shoot appears to be the predominant target of the biochemical and physiological effects. Studies using intact plants, isolated shoots and cell suspension cultures of *Galium* treated with the ethylene generator, ethephon, or inhibitors of ACC and ABA synthesis, suggested that quinmerac-stimulated ACC synthesis triggered the accumulation of ABA, mainly from de-novo synthesis.[3,4] In accordance, quinmerac did not induce ABA accumulation or greatly affect the growth of the wilt-prone flacca mutant of tomato, which is blocked at the final step of ABA biosynthesis.[5] Other quinmerac-susceptible weed species, such as *Apium graveolens* L., *Conium maculatum* L., *Solanum nigrum* L. and *Veronica* spp, also responded with an increase in ABA concentration which was closely correlated with reductions in root and shoot fresh weight.[5]

This raises the question of the contributions of ethylene and ABA in the mechanism of quinmerac selectivity. In the tolerant crop species sugarbeet, oilseed rape and wheat, quinmerac did not alter the formation of ethylene and the endogenous levels of ACC and ABA, when measured 48 h after treatment.[4] Carbon dioxide fixation and plant fresh weight were similarly unaffected. In addition, no notable differences in uptake and translocation of quinmerac between the crop species and *Galium* were detected 24 h after root application.[6] In wheat a more rapid metabolism of the herbicide was reported.[6] It was suggested that the main reason for the selective action of quinmerac is based on a different tissue/target sensitivity to the auxin herbicide in the different species.[2,4] Particularly, a selective interference with ACC synthase was hypothesized.[4] ACC synthase, the key enzyme in ethylene biosynthesis, is encoded by a multigene family with different ACC synthase genes being expressed in an organ-specific manner and in response to auxin.[7] However, the effects of quinmerac on ACC synthase activity have not, hitherto, been investigated. In addition, within the most important first 24 h of herbicide action, ethylene formation, ACC and ABA levels in the crop species have not been determined. Similarly, in this time period, data on uptake, translocation and metabolism of quinmerac are lacking.

Therefore, we studied the mechanism of quinmerac selectivity between *Galium* and sugarbeet, oilseed rape and wheat by directly comparing (i) the uptake, translocation and metabolism of the herbicide and (ii) the effects on ACC synthase activity, ethylene formation and the levels of ACC and ABA in short-term experiments.

2 MATERIALS AND METHODS

2.1 Chemicals

Quinmerac (7-chloro-3-methylquinoline-8-carboxylic acid; BAS 518H;[1] 99·4% pure; Fig. 1), [^{14}C]quinmerac ($>99\%$ radiochemical purity, sp.act. 438 MBq mmol^{-1}, 1·98 MBq mg^{-1}, labeled at C-2 atom in the heterocycle) and the plant metabolites of quinmerac, 7-chloro-3,8-quinoline dicarboxylic acid, (BAS 518-2) and 3-hydroxymethyl-7-chloro-quinoline-8-carboxylic acid (BAS 518-4) were synthesized at BASF Aktiengesellschaft, Ludwigshafen, Germany. 1-Aminocyclopropane-1-carboxylic acid was obtained from Calbiochem (Bad Soden, Germany), (+)- and (±)-*cis,trans*-abscisic acid from Sigma (Munich, Germany).

2.2 Cultivation of plants in hydroponics

Young plants of *Galium aparine* L., *Brassica napus* L. cv. Petranova, *Beta vulgaris* L. cv. Viktoria, and *Triticum aestivum* L. cv. Kanzler were raised to the second-leaf stage in vermiculite substrate moistened with 1/2 strength Linsmaier-Skoog[9] nutrient solution (light/dark: 16/8 h at 24/19°C, 250 μmol m^{-2} s^{-1}, 400–700 nm; fluorescent lamps, radium HRLV).[3] Uniformly developed plants were transferred into 320-ml glass vessels with 1/2 strength Linsmaier-Skoog medium and cultivated in growth chambers with 16/8 h light/dark cycles at 25/20°C and 75% relative humidity (3–12 plants per vessel, five replications; light: Osram Powerstar HQI-R 250 W/NDL and Osram Krypton 100-W lamps, *c.*530 μmol m^{-2} s^{-1}, 400–750 nm).[3] The solution was aerated throughout the experiments. After one day of adaptation, the compounds were added to the medium in acetone solution (1 ml litre^{-1}, final concentration) which had no adverse effect on plant growth. At various times after treatment, growth parameters were measured and shoots and roots from parallel vessels were harvested, immediately frozen in solid carbon dioxide and stored at -80°C. All experiments were repeated at least twice and proved to be reproducible. The results of a representative experiment are shown.

2.3 Determination of ethylene production

After treatment in hydroponic vessels, plants were transferred into 100-ml glass cylinders with 10 ml of 1/2 strength Linsmaier-Skoog medium (one plant per cylinder; six replications) as described previously.[3] The cylinders with plants were immediately sealed with

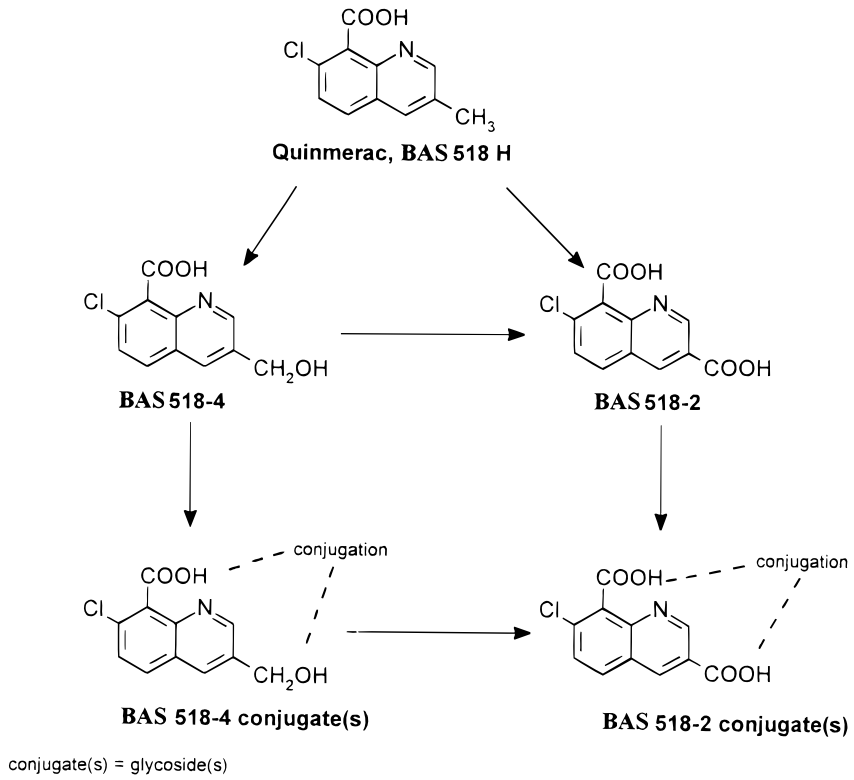

Fig. 1. Structural formula and metabolic pathway of quinmerac in plants according to Keller.[8]

rubber caps (Verneret, Pulheim, France) and incubated for a further 3 h at 25°C under light (*c.*55 μmol m^{-2} s^{-1}, 400–750 nm, Osram universal white neon tubes). At the indicated times, a 1-ml gas sample of the head space was withdrawn and ethylene was measured by gas chromatography using a Hewlett Packard GC Model 5890 (Hewlett-Packard, Waldbronn, Germany) equipped with an alumina column and a flame ionization detector. The column (1 m long, 3 mm diameter) was packed with alumina (60–80 mesh). Gas flow rates were: nitrogen 30 ml min^{-1}; hydrogen 30 ml min^{-1}; air 300 ml min^{-1}. The column temperature was 95°C, the injector temperature 150°C and the detector temperature 200°C.

2.4 Determination of ACC

Plant material was powdered under liquid nitrogen and samples (100 mg, three replications) were extracted in ethanol + water (70 + 30 by volume; 5 ml). After centrifugation (3500*g*, 10 min, 4°C), the supernatant was passed through a C_{18}-reversed-phase prepacked column (Seppak, Waters, Königstein, Germany) for purification. A 3-ml aliquot of the effluent was concentrated under vacuum to dryness, and brought to a volume of 2·75 ml with double-distilled water (2 ml) and potassium hydroxide solution (2 M; 0·75 ml). The ACC content was assayed by converting it directly to ethylene with sodium hypochlorite in the presence of mercury (II) chloride as described elsewhere.[3,10]

2.5 Determination of ABA

ABA was determined by immunoassay after extraction of powdered plant material (1 g; three replications) with methanol + water (80 + 20 by volume) containing 10 mg $litre^{-1}$ butylated hydroxytoluene followed by high performance liquid chromatographic (HPLC) separation on a reverse-phase Nucleosil 120-5 μm C_{18} column using a linear gradient from 5% aqueous methanol containing 0·1 M acetic acid to 100% methanol, as previously described.[11,12] Phytohormone concentration was quantified by enzyme-immunoassay with monoclonal antibodies 100% reactive against (+)ABA. The antibodies were kindly provided by Prof. E. W. Weiler (Univ. of Bochum). The data presented were corrected for recovery, which was about 50%, as determined using radiolabelled (+)ABA as internal standard added to the methanolic extract of the ground tissues.

2.6 Determination of ACC synthase activity

ACC synthase (EC 4.4.1.4.) was extracted and assayed as follows (Z.-H. Yin, C. Langebartels & H. Sandermann, GSF-Institut für Biochemische Pflanzenpathologie, Neuherberg, Oberschleißheim, Germany, pers. commun.). Powdered plant material (1·5 g) was homogenized in 100 mM EPPS (*N*-[2-hydroxyethyl]piperazine-*N*′-3-propanesulfonic acid)/potassium hydroxide buffer (pH 8·5) containing dithiothreitol (5 mM) pyridoxal phosphate (6 μM), leupeptin (10 μM) and Pefabloc SC (4-

(2-aminoethyl)phenylsulfonylfluoride hydrochloride, Merck, Darmstadt, Germany; 10 μM). The extract was centrifuged at 25 000*g* for 10 min (4°C) and the supernatant was passed through a Sephadex G25 column (Pharmacia, Uppsala, Sweden) which had been equilibrated with 5 mM EPPS buffer (pH 8·5) containing dithiothreitol (1 mM), pyridoxal phosphate (6 μM) and Pefabloc SC (10 μM). The ACC synthase assay mixture, with a total volume of 0·6 ml, contained 0·3 ml enzyme preparation in EPPS buffer (80 mM) with pyridoxal phosphate (20 μM) and *S*-adenosyl methionine (100 μM). After an incubation period of 2 h at 37°C, the reaction was stopped by adding mercury (II) chloride solution (20 μmol) and the ACC produced was determined by chemical conversion to ethylene as described above. All assays were performed in four replicates. For investigation of the effects of quinmerac on ACC synthase activity *in vitro*, ACC synthase was extracted from water-stressed shoot material (17% decrease in fresh weight) and 60 μl of an aqueous solution containing quinmerac prepared as a stock solution in dimethyl sulfoxide (50 g kg^{-1} final concentration in the assay) was added to the assay mixture before incubation.

2.7 Uptake and translocation of [^{14}C]quinmerac

Plants at the second leaf stage were placed in plastic vials (25 mm diameter, 38 mm height; Greiner, Nürtingen, Germany) with 10 ml of 1/2 strength Linsmaier-Skoog medium and the addition of 1 and 10 μM [^{14}C]quinmerac (4·38 and 43·8 kBq). The vials were closed with plastic covers with slits into which the plants were fitted upright (one to four plants per vial, representing similar plant fresh weights per species). Afterwards, the vials (five replicates) were placed in growth chambers (continuous light, 530 μmol m^{-2} s^{-1}, 25°C). At the indicated times, plants were removed from the medium and roots were carefully washed. Then, plants were sectioned into shoot and root, and fresh weights were determined. Plant parts were dried at 40°C for three days, combusted in a biological materials oxidizer (Oxymat OX 300, Zinsser, Frankfurt, Germany) and the evolved [^{14}C]carbon dioxide was absorbed in Oxosolve C-400 LS cocktail (Zinsser) and tested for radioactivity by scintillation counting.

2.8 Metabolism of [^{14}C]quinmerac

Plants at the second leaf stage were cultivated in glass vessels with 300 ml of 1/2 strength Linsmaier-Skoog medium in growth chambers as described above. After one day of adaptation, [^{14}C]quinmerac at 1 and 10 μM (0·13 and 1·3 MBq) was added to the medium and plants were harvested at 16 h after treatment. Shoot and root material was immediately frozen in solid carbon dioxide and stored at − 80°C. Plant material was homogenized in 100% methanol (two replications) with an Ultra-Turrax T 25 (IKA, Staufen, Germany). After centrifugation (5 min at 17 000*g*, Centrikon H-401, Kontron, Eching, Germany), the supernatant was concentrated to the aqueous phase by rotary evaporation and extracted three times with hexane. The aqueous extract was acidified with hydrochloric acid (1 M) to pH 2, and extracted three times with ethyl acetate. The combined ethyl acetate phases were concentrated to dryness by rotary evaporation and redissolved in acetonitrile + water (20 + 80 by volume; 100 μl). Then, 20 μl of the sample was separated by reverse-phase HPLC on a Spherisorb C_{18}-2 5-μm column (250 × 4 mm, Macherey-Nagel, Düren, Germany) using a linear gradient from 20% acetonitrile in water to 100% acetonitrile. The gradient sweep time was 20 min at a flow rate of 1 ml min^{-1}. Radioactivity of the fractions containing quinmerac (8·2 min retention time) and its metabolites BAS 518-2 (5·2 min) and BAS 518-4 (3·5 min) was determined using a HPLC coupled radioactivity monitor (LB 506 C, Berthold, Wildbad, Germany). As internal standard, [^{14}C]quinmerac was added to the methanolic extract of the homogenized material. Recovery was above 60% in all cases after the extraction and HPLC procedure. The metabolites BAS 518-2 and BAS 518-4 were identified by chromatographic mobility.

3 RESULTS AND DISCUSSION

3.1 Herbicidal selectivity

Young plants of *Galium*, oilseed rape, sugarbeet and wheat at the second leaf stage were tested for their reaction towards increasing concentrations of quinmerac (1 nM to 100 μM) applied hydroponically (Table 1). In order to quantify the selectivity between the weed and the tolerant crop species, the molar concentrations of quinmerac required for 30% reduction of shoot fresh

TABLE 1
Sensitivity of Young Plants of *Galium aparine*, Oilseed Rape, Sugarbeet and Wheat to Quinmerac after Root Treatment with Concentrations of 0·01 to 100 μM for Four Days

Plant species	*Concentrations required for 30% reduction of shoot fresh weight (IC_{30}) (μM)*	*SI*[a]
Galium aparine	0·07	
Brassica napus	30	429
Beta vulgaris	80	1143
Triticum aestivum	80	1143

[a] Selectivity index, SI = IC_{30} (crop)/IC_{30} (*Galium*).

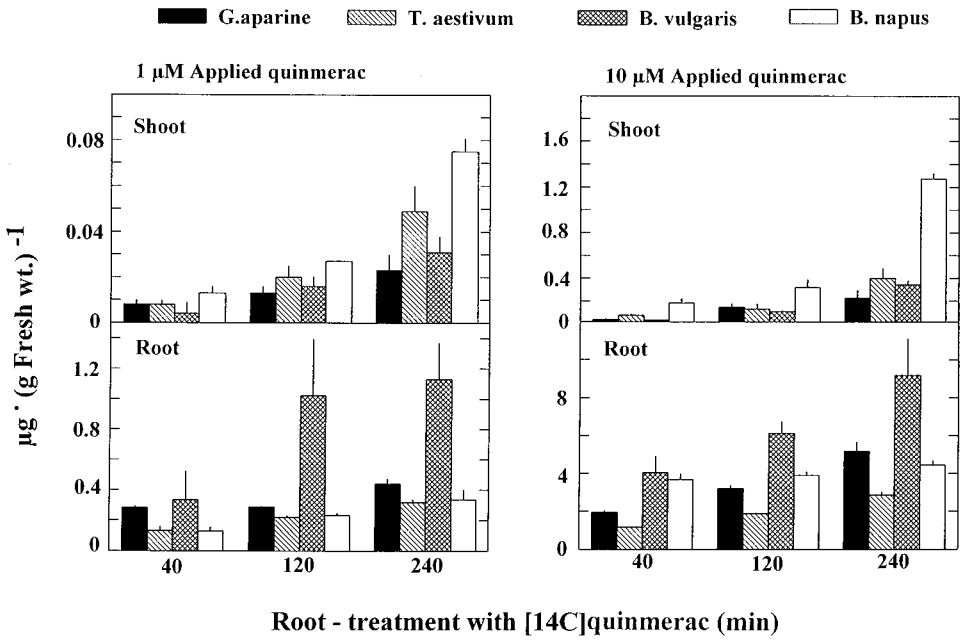

Fig. 2. Root-absorption and translocation of [^{14}C]quinmerac in *Galium aparine*, oilseed rape, sugarbeet and wheat. Plants at the second leaf stage were treated hydroponically with 1 and 10 μM [^{14}C]quinmerac for the indicated times (five replicates). After incubation, radioactivity was determined in the plant organs. On the basis of the specific activity of the [^{14}C]quinmerac (1·98 KBq μg^{-1}), the concentration of the compound was related to fresh weight. Vertical bars represent SE of the mean.

weight (IC_{30} values) were calculated from linear regression equations (Table 1). As shown in Table 1, the selectivity index of quinmerac between oilseed rape and *Galium* (SI) was 429 and that between sugarbeet or wheat and *Galium* was 1143.

3.2 Absorption, translocation and metabolism of [^{14}C]quinmerac

After hydroponic treatment of *Galium* plants with 1 and 10 μM [^{14}C]quinmerac, root uptake increased during

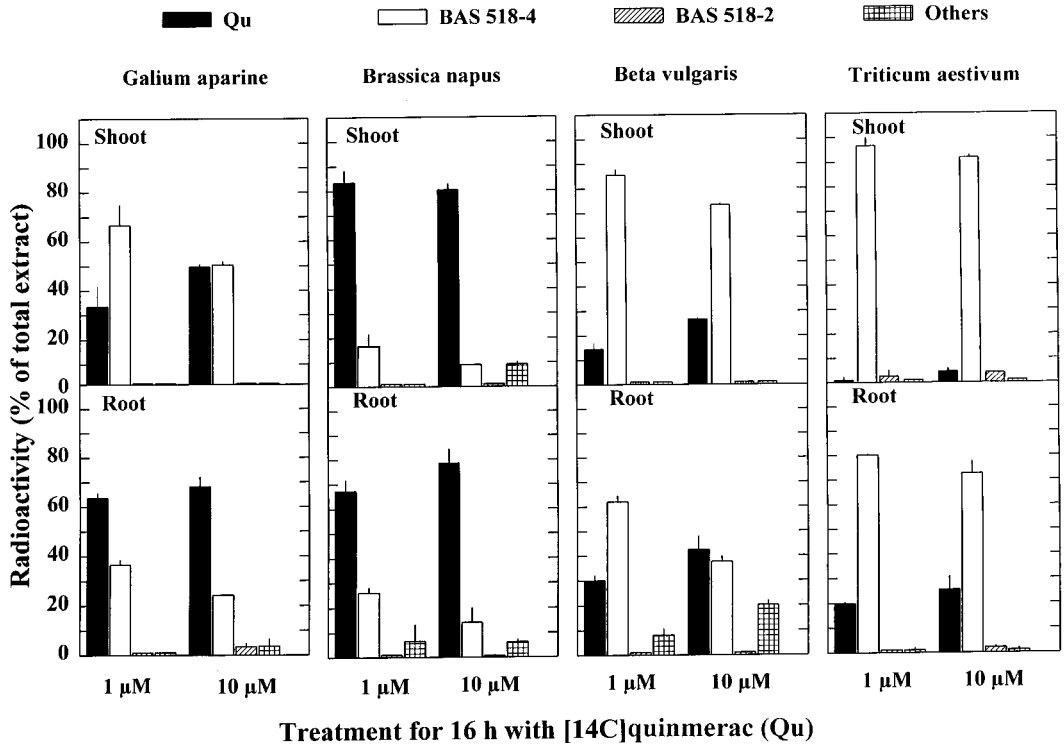

Fig. 3. Metabolism of [^{14}C]quinmerac in *Galium aparine*, oilseed rape, sugarbeet and wheat. Plants at the second leaf stage were root-treated with 1 and 10 μM [^{14}C]quinmerac. After incubation for 16 h, radioactivity was extracted from shoots and roots (three replicates) and analysed by HPLC-radiocounting. The metabolites of quinmerac (QU), BAS 518-2 (dicarboxylic acid) and BAS 518-4 (hydroxymethyl derivative) were identified by chromatographic mobility. Vertical bars represent SE of the mean.

the 4 h incubation period (Fig. 2). In this time interval, approximately 8% of the applied radioactivity was absorbed by the roots and translocated, in part, into the shoot. Assuming that water constitutes 90% of tissue fresh weight, this represents root tissue [^{14}C]-compound concentrations of 2·2 and 26 μM, at 1 and 10 μM applied quinmerac, respectively. Based on root fresh weight, lower concentrations of [^{14}C]quinmerac were found in wheat than in *Galium* (Fig. 2), although a slightly higher proportion (9%) of the applied radioactivity was taken up by the crop species within the incubation period. In oilseed rape, uptake and calculated compound concentrations in the root were similar to those found in *Galium*. By contrast, in the roots of sugarbeet, 13% of the applied radioactivity was absorbed and nearly two-fold higher concentrations of radiolabelled compound were found, compared to *Galium*. Following root treatment with 1 or 10 μM [^{14}C]quinmerac of *Galium*, wheat and sugarbeet plants for 4 h, approximately 15%, 24% and 21% of the absorbed ^{14}C was translocated acropetally to the shoot, respectively. Based on fresh weight, the amounts of [^{14}C]-compound in the shoot tissue of the various plant species differed in the same proportion (Fig. 2). In *Galium* shoots, endogenous concentrations of 0·12 and 1·2 μM were calculated after exposure to 1 and 10 μM [^{14}C]quinmerac for 4 h, respectively. In the shoot tissue of oilseed rape, quinmerac accumulated to approximately two- to three-fold higher concentrations compared to those of the other plant species (Fig. 2). In this crop, 72% of the root-absorbed ^{14}C was translocated into the shoot.

More distinct differences were found in the metabolism of quinmerac in the plant species tested (Fig. 3). In these experiments, plants were root-treated with 1 and 10 μM [^{14}C]-quinmerac for 16 h. At 10 μM applied [^{14}C]quinmerac, approximately 80, 50, 27 and 4% of the absorbed radioactivity was found as unchanged herbicide in the shoot tissues of oilseed rape, *Galium*, sugarbeet and wheat, respectively. The main metabolite was the hydroxymethyl-derivative BAS 518-4 which is further oxidized to the dicarboxylic acid BAS 518-2 (Fig. 1). When applied to the plant species, both metabolites were biologically inactive (data not shown). In the root tissue, the portion of unchanged parent was mostly higher than in the shoot (Fig. 3), probably due to the continuous root uptake of [^{14}C]quinmerac. Similar results were obtained after application of 1 μM [^{14}C]quinmerac (Fig. 3). Consequently, compared to *Galium*, a more rapid metabolism of quinmerac in sugarbeet and particularly in wheat could contribute to the selectivity of the herbicide. In contrast, in the shoots of oilseed rape susceptibility to the herbicide is enhanced by the higher quinmerac accumulations and the lower rate of metabolism, compared to *Galium* and the other crop species. Accordingly, sensitivity of oilseed rape to quinmerac is higher than that of sugarbeet or wheat (Table 1). However, oilseed rape was found to be nearly 400-fold less susceptible to quinmerac than *Galium* (Table 1). In conclusion, the selective action of quinmerac is not primarily based upon differences in uptake, translocation, or metabolism of the herbicide.

3.3 Short-term effects on ethylene biosynthesis and abscisic acid levels

In sensitive dicotyledonous weeds such as *Galium*, a dramatic accumulation of ABA, induced ultimately through quinmerac-stimulated ethylene biosynthesis, is implicated as the mode of action underlying the inhibition of growth.[3–5] Within 1 h of root-treatment of *Galium* plants with quinmerac, concentration-dependent increases in ACC synthase activity and ACC levels in the shoot tissue were induced (Fig. 4A). During the incubation period, ACC synthase activity was increased up to 50-fold, relative to the control, at 10 μM quin-

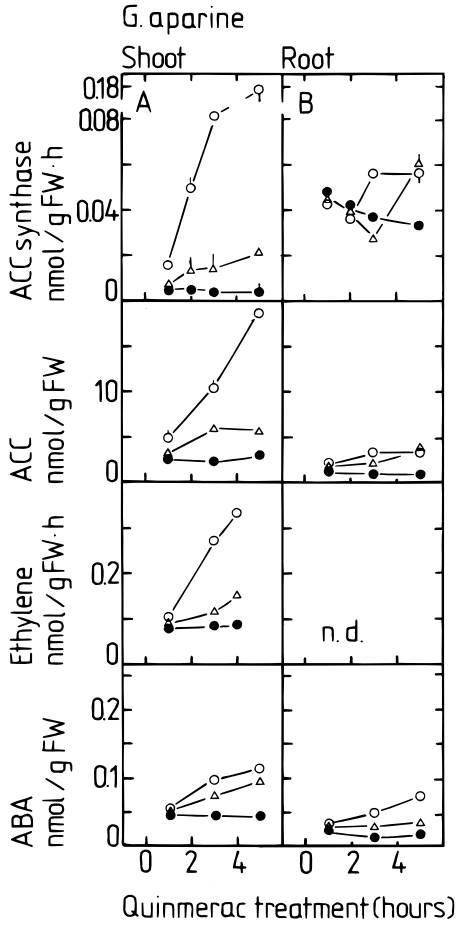

Fig. 4. Time course of the effects of quinmerac on ACC synthase activity, ethylene formation and the contents of ACC and immunoreactive ABA in (A) shoot and (B) root of *Galium aparine*. Plants at the second leaf stage were root-treated with quinmerac hydroponically. (●) Control, (△) 1 μM, (○) 10 μM quinmerac. n.d., not determined. Vertical bars represent SE of the mean.

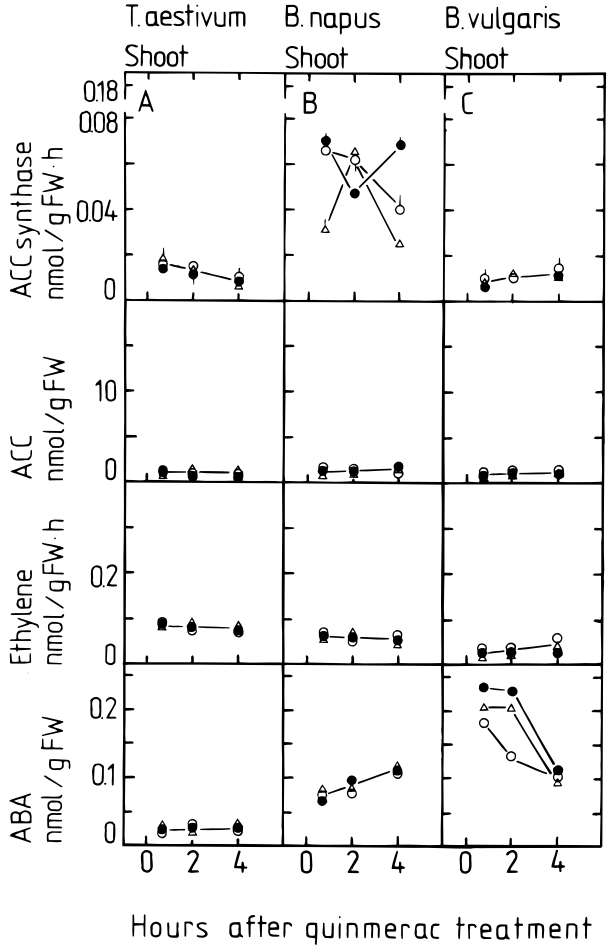

Fig. 5. Time course of the effects of quinmerac on ACC synthase activity, ethylene formation and the contents of ACC and immunoreactive ABA in shoots of (A) wheat, (B) oilseed rape and (C) sugarbeet. Plants at the second leaf stage were root-treated with quinmerac hydroponically. (●) Control, (△) 1 μM, (○) 10 μM quinmerac. Vertical bars represent SE of the mean.

merac. Within 3 h, rises in ethylene formation and ABA concentrations followed (Fig. 4A). In the root tissue, ACC and ABA levels showed lower increases and ACC synthase activity was only slightly changed during the incubation period (Fig. 4B). Hence, quinmerac elicits ACC synthase activity and ABA accumulation specifically in the shoot tissue. After 48 h, levels of ABA were elevated up to 70-fold, relative to untreated plants.[3] The ACC and ABA produced appear to be translocated basipetally to the root. This conclusion was confirmed by recent experiments with detached shoots and roots of *Galium* treated *in vitro*.[3] After quinmerac exposure, only isolated shoots showed a significant stimulation of ethylene biosynthesis and ABA content, whereas in roots the concentrations of these metabolites remained unchanged. In addition, treatment of isolated roots with ACC or ethephon did not cause ABA accumulation (unpublished results). Hence, the rise in ABA found in the roots of herbicide-treated *Galium* plants was neither triggered by quinmerac nor indirectly by ACC from the shoot. It appears to be the result of ABA transport from the shoot. In contrast to *Galium* plants, in the shoots of wheat (Fig. 5A), oilseed rape (Fig. 5B) and sugarbeet (Fig. 5C), quinmerac did not stimulate ACC synthase activity, ethylene formation and ACC and ABA levels. Therefore, it is suggested that the main reason for the selectivity of quinmerac lies in the lower sensitivity to the herbicide of the site of action. Since quinmerac, added in concentrations of 0·1 to 100 μM, did not alter the *in-vitro* activity of ACC synthase extracted from detached shoots of *Galium*, the induction process of the ACC synthase activity is proposed as the primary target of compound interference. As discussed for other auxin herbicides,[13] affinity of quinmerac for auxin-binding sites or signal transduction processes leading to *de-novo* synthesis of ACC synthase could be the mechanism of action.

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